KIS (KChIP4aΔ2-34) demonstrated that KChIP4a with KIS domain had no significant effect on steady-state activation, but shifted the voltage dependence of steady-state inactivation of Kv4.3 to hyperpolarizing direction by enhancing closed-state inactivation. We have previously demonstrated that KChIP4a can rescue the function of a tetramerization-defect mutant Kv4.3 C110A. The rescued Kv4.3 C110A current is larger than that of WT Kv4.3/KChIP4a co-expression, although C110A surface expression was lower. Upon coexpression, the closed-state inactivation of Kv4.3 C110A mutant was less affected by the KIS domain, as compared with enhanced closed-state inactivation observed in wild-type channel, suggesting a role of T1 domain in mediation of Kv4 closed-state inactivation. Taken together, we propose that N-terminal KIS domain of KChIP4a inhibits Kv4.3 function through dual independent mechanisms by which auxiliary KChIP4a causes Kv4 ER retention and promotes channel closed-state inactivation.

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Targeted Mutagenesis with a Homology Model Identifies Critical Residues for Arachidonic Acid Inhibition of Kv4 Channels

Linda M. Boland[1], Robert Heler[1], Jessica A. Bell[2].
[1]University of Richmond, Richmond, VA, USA, [2]Virginia Commonwealth University, Richmond, VA, USA.

Polyunsaturated fatty acids such as arachidonic acid (AA) demonstrate inhibitory modulation of Kv4 potassium channels. Molecular docking approaches using a new Kv4.2 homology model predicted a membrane-embedded binding pocket for AA comprised of the S4-S5 linker on one subunit and several nearby hydrophobic residues within the internal side of S5 and S6 from an adjacent subunit. We tested the hypothesis that modulatory effects of AA on Kv4.2/KChIP channels require access to this site. Targeted mutation of a lysine residue and a nonpolar residue within the S4-S5 linker as well as a nonpolar residue in S3 significantly impaired the effects of AA on K^+ currents in *Xenopus* oocytes. These residues may be important in stabilizing or regulating access to the negatively charged carboxylate moiety on the fatty acid. The structural specificity of this interaction was supported by the lack of disruption of AA effects observed with charge neutralizing mutations at residues located near but not within the predicted binding pocket. Furthermore, we found that the crystal structure of the Kv1.2/2.1 channel chimera lacks an AA docking site with the structural features present in the proposed hydrophobic pocket of Kv4.2 and the chimera was likewise unaffected by AA. We simulated the mutagenic substitutions of critical residues identified in our Kv4.2 model to provide a structural interpretation of the disruption of the AA binding pocket. We conclude that AA inhibits Kv4 channel currents and facilitates inactivation by interacting with a hydrophobic binding pocket in which a lysine residue within the S4-S5 linker is important for AA interaction.

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Modification of Kv4.2 Channel Complexes by the Diphenylurea Compound NS5806

Katrin Witzel, Regina Westermeier, Paul Fischer, **Robert Bähring**.
UKE, Hamburg, Germany.

NS5806 (N-[3,5-bis(trifluoromethyl)phenyl]-N-[2,4-dibromo-6-(1H-tetrazol-5-yl)phenyl]-urea) can increase the amplitude and slow the decay of currents mediated by voltage-gated potassium (Kv) channels of the Kv4 subfamily if Kv Channel Interacting Proteins (KChIPs) participate in channel complex formation. We studied the effects of NS5806 on the somatodendritic A-type current (Isa) in hippocampal neurons and on the currents mediated by Kv4.2 channels coexpressed with different auxilliary beta-subunits in HEK 293 cells with the whole-cell patch-clamp technique. The effects of NS5806 on the inactivation gating of Kv4.2 mutants were studied under two-electrode voltage-clamp in Xenopus oocytes. Notably, the Isa component in hippocampal neurons was reduced rather than potentiated, but showed a pronounced slowing of inactivation in the presence of NS5806. The peak amplitudes of currents mediated by ternary Kv4.2 channels, coexpressed with different KChIPs and dipeptidyl aminopeptidase-related protein (DPP) 6 were potentiated and their macroscopic inactivation slowed by NS5806. The currents mediated by binary Kv4.2 channels, coexpressed only with DPP6, were suppressed by NS5806 and the effects on macroscopic inactivation were less pronounced. Similar to the Isa component in hippocampal neurons, the midpoint voltage of inactivation of recombinant Kv4.2 channel complexes was shifted negative by NS5806. However, the recovery from inactivation was accelerated in hippocampal neurons and slowed in recombinant channels. Functional analysis of Kv4.2 S4S5 linker and S6 mutants support the notion that NS5806 influences the dynamic coupling between voltage sensor and gate.

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Mechanism of Inhibition of Voltage-Gated Potassium Channels by Guanidine Compounds

Jeet Kalia, Kenton J. Swartz.
NINDS, NIH, Bethesda, MD, USA.

Inhibition of presynaptic voltage-gated potassium (Kv) channels by guanidine underlies its use in the therapy for the neuromuscular diseases, myasthenic syndrome of Lambert-Eaton and botulism. The therapeutic use of guanidine is limited, however, due to side effects that accompany its administration. Therefore, the design of guanidine analogs with improved therapeutic indices is desirable. Progress towards this goal is hindered by the lack of knowledge of the mechanism by which these molecules inhibit Kv channels. We examined an array of possible mechanisms of inhibition of the Shaker Kv channel by guanidine, methyl guanidine and *N*,*N*-dimethyl guanidine, including charge screening, disruption of the protein-lipid interfaces, direct interaction with the voltage sensors and pore-binding. Our results demonstrate that guanidines bind within the intracellular pore of the channel, and perturb a hydrophobic subunit interface to stabilize a closed state of the channel. This mechanism provides a foundation for the design of guanidine analogs for the therapeutic intervention of neuromuscular diseases.

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Sevoflurane: A Potent General Anesthetic Enhancer of Kv Channel Activation

Annika F. Barber, Qiansheng Liang, Manuel Covarrubias.
Thomas Jefferson University, Philadelphia, PA, USA.

General anesthetics have been shown to both inhibit and potentiate various families of ion channels. However, the molecular bases of these actions are not understood. Previous work with K-Shaw2 explored structural determinants of inhibition by alkanols and the volatile general anesthetics halothane and isoflurane (Barber et al. Biophys J. 2011, in press). In this study, we report that sevoflurane, an inhaled anesthetic currently used in human general anesthesia, exhibits the singular ability to reversibly potentiate K-Shaw2 currents investigated in Xenopus oocytes under two-electrode voltage-clamp conditions (~80% potentiation at 1 mM). This result is in sharp contrast to the action of multiple other inhaled (halothane, isoflurane, desflurane, chloroform, and 1-alkanols) and injected (propofol) general anesthetics, which are inhibitory. This potentiation occurs over a clinically relevant concentration range (0.05 - 1 mM) in a dose-dependent manner that suggests two binding sites with distinct apparent affinities: a high-affinity site (K1 = 60 μM) and a low affinity site (K2 = 4 mM). Furthermore, a double mutation involving the S6 segment and the S4-S5 linker (A326V/A417V) abolishes the potentiation by sevoflurane. This effect is especially interesting because this double mutation also abolishes the inhibitory action of halothane, suggesting that these inhaled anesthetics share effector sites but influence gating in opposite ways. Semiquantitative kinetic modeling suggests that preferential binding of sevoflurane to resting and activated closed states shifts the channels into a novel gating mode with an enhanced open probability. Additional screening of related voltage-gated K channels at a various doses of sevoflurane revealed only modest potentiation of Kv1.2 no effect on Kv1.3, Kv2.1, Kv3.4 and Kv4.2. This study provides a novel framework for further investigations of the structural basis of general anesthetic action.

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Activation and Inactivation Kinetics of the Potassium Channels K_V1.3 and K_V1.5 Measured on the CytoPatch™ Instrument and the Manual Patch Clamp: A Comparative Study

Olaf Scheel, Gesa Rascher-Eggstein, Thomas Knott.
CYTOCENTRICS AG, Rostock, Germany.

The state of the art method to study ion channels is the patch clamp technology. Since 2003, patch clamp automats offered an efficiently faster analysis method, as it would have been feasible with the manual patch clamp technique. Nevertheless, the advantage of higher throughput in patch clamp automation is accompanied with a lower data quality.
The CytoPatch™ Instrument is a patch clamp automat with a new chip technology, the Cytocentering Technology. With this, the automat is capable to generate a data quality and flexibility comparable to the manual patch clamp technology. To investigate this issue we carried out a comparative study on the activation and inactivation kinetics of the potassium channels K_V1.3 and K_V1.5 measured on the CytoPatch™ Instrument and the manual patch clamp. The data prove that advanced electrophysiological studies can be carried out on the CytoPatch™ Instrument with similar results to the manual patch clamp technique. Furthermore, it goes along with the benefit of automation which is: a higher standardisation - walk away times of several hours - automated data evaluation - no need of a highly-skilled person to run the instrument - higher throughput and lower costs per data point.